J. Phytopathology **153**, 269–273 (2005)

Crop Science Department, Faculty of Agriculture, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey

Reaction of Cotton Cultivars and an F_2 Population to Stem Inoculation with Isolates *Verticillium dahliae*

Y. Bolek[1], A. A. Bell[2], K. M. El-Zik[3], P. M. Thaxton[3] and C. W. Magill[4]

Authors' addresses: [1]Crop Science Department, Faculty of Agriculture, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey; [2]USDA-ARS, College Station, TX, USA; [3]Soil and Crop Sciences, Texas University, College Station, TX, USA; [4]Plant Pathology, Texas University, College Station, TX, USA (correspondence to Y. Bolek. E-mail: yuksel@ksu.edu.tr)

Received December 1, 2004; accepted March 8, 2005

Keywords: Gossypium, Verticillium wilt, cotton, *Verticillium dahliae*, resistance

Abstract

Four *Verticillium dahliae* isolates (V76, TS-2, PH, and V44) were used in screening four cotton cultivars (Pima S-7, Acala Prema, M-315 and Acala 44). Pima S-7 and Acala Prema gave the highest resistance reactions and Acala 44 was the most susceptible. Isolate V76 of *V. dahliae* was the most virulent. An interspecific cross between the resistant cv Pima S-7 (*Gossypium barbadense*) and the susceptible cv. Acala 44 (*G. hirsutum*) was made and the F_2 population phenotyped for Verticillium wilt effect. Phenotyping of plant reaction to the disease was quantified by using a set of six growth parameters (number of healthy leaves, number of nodes, leaf weight, stem weight, leaf to stem ratio, and total shoot weight) measured 3 weeks after inoculation. The F_2 phenotypic distribution of these parameters suggests that distribution is towards resistance and polygenic. Transgressive segregation also was observed. The number of healthy leaves and total shoot weight were found to be the best indicators of resistance. Results obtained in this study will be useful to quantify resistance to *V. dahliae* and identify the best parameters to phenotype in genetic studies.

Introduction

One of the major constraints affecting yield and quality of cotton is disease. Verticillium wilt incited by the soil inhabiting fungus *Verticillium dahliae* Kleb., is found in many regions of the world where cotton is produced, and it can cause substantial yield losses. Verticillium wilt was first reported in 1914 in Virginia (Carpenter, 1914), and first recognized as an economically important disease of cotton in 1927 in Tennessee (Sherbakoff, 1928). It has been recognized as one of the major diseases of cotton in many cotton-producing countries: Australia, Brazil, Bulgaria, China, Greece, Peru, Turkey, Uganda, USA and Uzbekistan (Adair, 1996). Yield losses in the USA have ranged from 0.75 to 2.78% during the past 20 years (National Cotton Council, 1980–1999), and losses in other parts of the world have been as high as 30% (Bell, 1992). The report of the Cotton Disease Loss Committee for the 2003 crop indicated that losses across the cotton belt averaged 0.42% (ranging from no loss to as high as 4.0% in different states) with an estimated yield loss of 71 687 bales (Blasingame and Patel, 2004).

Vegetative compatibility (V-C) tests and isozyme analyses have been used to show that there are four genetically isolated populations within *V. dahliae*, and that each V-C group has at least two subgroups (Bell, 1994). Isolates which fall into VCG 1 are known as defoliating whereas isolates VCG 2 are non-defoliating.

The severity of Verticillium wilt depends upon the inoculum density, virulence of the pathogen, the cultivar (genetic constitution, age and physiological condition), temperature, soil conditions (pH, moisture and nutrient availability) and biological antagonists (Bell, 1993). An integrated management system is necessary to minimize losses from the disease (El-Zik, 1985). Genetic resistance can be selected within adapted desirable cultivars with a suitable breeding programme. Modern Upland cultivars, such as Acala Maxxa, Acala Prema, Acala Royale, as well as *Gossypium barbadense* and Pima cultivars, are highly tolerant to wilt, while Deltapine 20, Deltapine 51, Deltapine 5690, Stoneville 495, Hyperformer HS-23 and Paymester HS-26 (*G. hirsutum*) have moderate tolerance and effectively control disease in areas where inoculum density is moderate (Bell, 1999).

Specific objectives were to determine levels of resistance and susceptibility in four cotton cultivars and virulence of four *V. dahliae* isolates, and the best plant parameters to indicate resistance.

Materials and Methods

Parental selection

Cotton genotypes and pathogen isolates Four cotton cultivars Pima S-7 (*G. barbadense*), Acala 44, M-315 and Acala Prema (*G. hirsutum*); and four *V. dahliae* isolates V76 (VCG-1), PH, TS-2 and V44 (VCG-2) were selected for this study. The experiment was conducted in an environmentally controlled growth chamber. Thirty-six plants per cultivar and nine plants per isolate were inoculated in a randomized factorial design to identify resistant and susceptible cultivars and their reaction to the four isolates of *V. dahliae*.

Inoculation and phenotyping Acid-delinted seeds of each cultivar were germinated in paper rolls moistened with tap water at 30°C for 24-h and then transferred to 16 ounce plastic cups having three holes on the bottom for drainage and filled with potting soil and grown in the greenhouse. After expansion of 5–6 true leaves, the plants were moved to an environmental growth chamber with a 12-h lighted day temperature of 27°C and dark night temperature of 22°C. This is the optimum temperature for virulence expression for all isolates tested (A. A. Bell, personal communication). Plants were allowed to equilibrate for at least 1 week at these conditions before inoculation.

Verticillium dahliae isolates were grown on potato dextrose agar plates at room temperature (23°C). For inoculum preparation, a conidial suspension was spread on plates that were incubated at 25°C for 3–4 days, and then conidia were collected, washed with sterile water, and diluted to a concentration of $2–5 \times 10^7$ cells/ml and used for inoculation.

Plants were stem inoculated immediately below the cotyledonary nodes at two sites with the stem puncture technique using syringe and needle (Bugbee and Presley, 1967). Three weeks after inoculation, data was collected and disease reactions were scored as described by Devey and Rosielle (1986), and Hillocks (1990). The number of healthy leaves is the number of leaves larger than 2 cm in diameter from the sides that are fully green or have remained attached to the main stem of the plant and they are not malformed or wilted. The petiol was not included in the leaf measurement. The number of nodes per plant was counted from the cotyledonary node to the top of the plant. Leaves taken off from both the main stem and secondary branches with their petioles attached were used to determine leaf weight. Stem weight was measured by weighing the stem that was cut at the cotyledon node and stripped of leaves and fruits. After these measurements, leaf–stem ratio and total shoot weight were calculated from leaf weight and stem weight.

Data analysis Data collected for the six previously mentioned traits to indicate resistance and susceptibility from disease screening were analysed statistically using analysis of variance (comparing the four cotton cultivars and the four *V. dahliae* isolates over the test) in order to calculate *F*-values and correlation coefficients (SAS Institute, 1994). LSD was performed to separate mean values.

F_2 population analysis

Mating and field plot design In order to maximize phenotypic expression and molecular polymorphism with respect to Verticillium wilt resistance/susceptibility, an interspecific cross was made between chosen parents: Pima S-7 (*G. barbadense*) and Acala 44 (*G. hirsutum*). The F_1 progeny was grown in the greenhouse to produce F_2 seed and in environmental growth chambers to assay for disease resistance. An F_2 population consisting of 110 individuals was used for the genomic study. The F_2 plants were derived from a single self-pollinated F_1 individual (single seed descent) obtained from the interspecific cross of the chosen parental plants that were pure breeding for reaction to Verticillium wilt.

Inoculation and phenotyping The most virulent defoliating isolate, *V. dahliae* (V76), was used to inoculate the parents and the 110 individual F_2 plants. The same procedures were followed as in parental selection to screen the effects of disease on the F_2 population.

Data analysis Frequency distribution histograms with parental mean values, correlations, chi-square tests and broad sense heritability estimates were derived to determine inheritance patterns and gene action.

Broad-sense heritability was estimated according to the equation $H^2 = \text{VarG}/\text{VarP}$, where VarP is phenotypic variance in F_2.

Results and Discussion

Parental selection

Analysis of variance for traits associated with resistance to Verticillium wilt of cotton showed significant differences exist among cotton cultivars and *V. dahliae* isolates (Table 1). Number of healthy leaves, leaf weight and leaf–stem ratio also had significant cultivar × isolate interaction (Table 1a,b,e). No significant replication effect was detected. Number of nodes and stem weight showed no significant difference among pathogen isolates (Table 1b,d).

The LSD tests comparing mean values of cultivars inoculated with different *V. dahliae* isolates (Table 2) show that Pima S-7 (resistant) and Acala Prema (resistant) had the highest mean values for each individual trait, while average mean value for Acala 44 (susceptible) was significantly lower than the other cultivars for all factors except the leaf–stem ratio. Mean values for M-315 were always in between those of Acala Prema and Acala 44. Mean values of Pima S-7 and Acala Prema for leaf weight and total shoot weight, and mean leaf–stem ratio of M-315 and Acala 44 were very close to each other and not significantly different, while in other traits all cultivars had different response to isolates. Mean difference was the greatest

Table 1
Analysis of variance of four cotton cultivars for traits associated with resistance to Verticillium wilt

Source	d.f.	SS	MS	*F*-value	P > *F*
(a) Number of healthy leaves					
Replication	2	1.43	0.72	0.29	ns
Cultivar	3	260.39	86.80	34.79****	< 0.0001
Isolate	3	165.17	55.06	22.07****	< 0.0001
C*I	9	67.22	7.47	2.99**	0.0029
Error	126	314.35	2.49		
Corrected total	143	808.56			
(b) Number of nodes					
Replication	2	2.54	1.27	0.67	ns
Cultivar	3	135.33	45.11	23.63**	< 0.0001
Isolate	3	6.83	2.28	1.19	ns
C*I	9	25.72	2.86	1.50	ns
Error	126	240.57	1.91		
Corrected total	143	411.00			
(c) Leaf weight					
Replication	2	29.91	14.95	1.54	ns
Cultivar	3	700.97	233.66	24.01****	< 0.0001
Isolate	3	293.88	97.96	10.07****	< 0.0001
C*I	9	204.38	22.71	2.33*	0.0182
Error	126	1126.11	9.73		
Corrected total	143	2455.25			
(d) Stem weight					
Replication	2	28.79	14.40	2.59	ns
Cultivar	3	340.59	113.53	20.45****	< 0.0001
Isolate	3	32.37	10.79	1.94	ns
C*I	9	92.64	10.29	1.85	ns
Error	126	699.66	5.55		
Corrected total	143	1194.05			
(e) Leaf–stem ratio					
Replication	2	0.14	0.07	0.85	ns
Cultivar	3	3.67	1.22	14.9****	< 0.0001
Isolate	3	5.17	1.72	20.98****	< 0.0001
C*I	9	4.23	0.47	5.73****	< 0.0001
Error	126	10.34	0.08		
Corrected total	143	23.54			
(f) Total shoot weight					
Replication	2	113.26	56.63	2.10	ns
Cultivar	3	1936.25	645.42	23.94****	< 0.0001
Isolate	3	478.21	159.40	5.91**	0.0018
C*I	9	435.20	48.36	1.79	ns
Error	126	3397.43	26.96		
Corrected total	143	6360.34			

*0.05, **0.01 and ****0.0001 significant at probability levels.

in total shoot weight; Pima S-7 was 19.29 g vs. 9.82 g for Acala 44.

In the analysis of *V. dahliae* isolates over cotton cultivars for the traits measured (Table 3), V76 was significantly different from the other isolates in terms of disease symptoms and damage to the plants. Of six traits, only number of nodes did not show any significant difference among isolates. The difference between the most virulent and least virulent isolates ranged from 12.87 g to 16.38 g in total shoot weight.

There were very strong and significant correlations as the 0.0001 probability level exist among traits over all isolates and cultivars. Based on all analyses, Pima S-7 (resistant) and Acala 44 (susceptible) were chosen as parents to develop an F_2 population for molecular studies and V76 was chosen as the *V. dahliae* isolate for disease screening.

Gossypium hirsutum cv Acala 44 is an Upland cotton cultivar, which is highly susceptible to *V. dahliae*. It is severely stunted and wilted by non-defoliating strains and is almost completely defoliated by defoliating strains. *Gossypium barbadense* cv. Pima S-7 is lower yielding cotton with high fibre quality, interspecifically compatible to *G. hirsutum*, and resistant to the Verticillium wilt pathogen. It exhibits transient minor symptoms with non-defoliating strains and only partial defoliation and some stunting with defoliating strains.

F_2 population analysis

Analysis of the F_2 population indicated that Verticillium wilt resistance is a quantitative trait with normal distribution for all traits (Fig. 1). Transgressive segregation towards increased resistance was observed. Curves were shifted towards resistance indicating a dominant component for resistance is present. Because of the effect of the most virulent isolate V76 on number of healthy leaves, leaf weight and leaf–stem ratio, minimum values measured were zero. The widest range of variation was observed in total shoot weight. Broad-sense heritability ranged from 0.58 to 0.88 for traits measured (Table 4).

Conclusion

To identify and select cotton cultivars and *V. dahliae* isolates for developing a segregating population for molecular analysis, four cultivars (Pima S-7, Acala Prema, M-315 and Acala 44) and four *V. dahliae* isolates (V44, PH, TS-2, V76) were screened for disease resistance. Traits chosen for the study are direct measurements of different phenological characteristics of cotton, and have a relationship with resistance and susceptibility to the pathogen. A relatively higher number of healthy leaves, main stem nodes, leaf weight, or stem weight indicate resistance. Leaf to stem ratio and total shoot weight are calculated from direct measure-

Table 2
Mean values of four cotton cultivars following inoculation with four *V. dahliae* isolates for traits associated with resistance to Verticillium wilt

Cultivar	N	Number of H. leaves	Number of nodes	Leaf weight (g)	Stem weight (g)	Leaf–stem (ratio)	Total shoot weight (g)
Pima S-7	36	6.31a	11.36a	9.41a	9.88a	0.94b	19.29a
Acala Prema	36	5.44b	10.53b	9.58a	8.63b	1.10a	18.21a
M-315	36	4.81b	10.42b	7.06b	8.54b	0.77c	15.59b
Acala 44	36	2.67c	8.69c	4.14c	5.68c	0.68c	9.82c
Mean		4.81	10.25	7.55	8.18	0.88	15.73
CV%		17.00	10.36	28.40	24.96	17.13	25.69

Mean values with the same letter within a column are not significantly different at the 0.05 probability level by LSD.

Table 3
Mean values of four *V. dahliae* isolates over four cotton cultivars for traits associated with resistance to the pathogen

Cultivar	N	Number of H. leaves	Number of nodes	Leaf weight (g)	Stem weight (g)	Leaf–stem (ratio)	Total shoot weight (g)
V44	36	5.06a	10.28a	7.96a	8.33ab	0.93a	16.38a
PH	36	5.75a	10.53a	9.00a	8.89a	1.02a	17.89a
TS-2	36	5.42a	10.28a	8.06a	7.81ab	1.00a	15.77a
V76	36	3.00b	9.92a	5.17b	7.69b	0.55b	12.87b
Mean		4.81	10.25	7.55	8.18	0.88	15.73
CV%		25.67	11.56	34.27	28.60	22.81	30.79

Mean values with the same letter within a column are not significantly different at the 0.05 probability level by LSD.

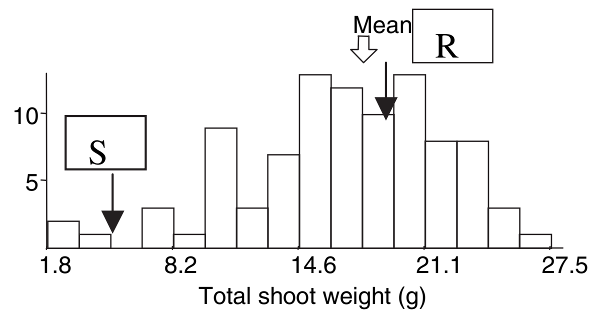

Fig. 1 Frequency distribution of total shoot weight associated with response to Verticillium wilt in the F_2 population three weeks after inoculation. Average values for resistant (R) and susceptible (S) parents are indicated by black arrows while white arrow used to indicate F_2 mean

ments of leaf and stem weight. A high ratio and high total shoot weight indicate resistance. Comparison of mean values for traits measured showed that significant differences exist among cotton cultivars and isolates. The differences were greatest between Pima S-7 and Acala 44. In addition, V76 was more virulent than the other isolates in terms of disease severity. It was chosen as an isolate to screen the F_2 population because it yielded significant differences between resistant and susceptible cultivars.

Leaf weight and total shoot weight of Pima S-7 and Acala Prema were not significantly different, indicating similar resistance level to wilt. Acala Prema is reported as resistant to Verticillium wilt (Bell, 1992). M-315 is resistant to Fusarium wilt but only tolerant to Verticillium wilt. Number of nodes over cultivars had no significant differences in V76 infected plants. This may be the result of damage being manifested less in nodes than the leaves which show symptoms and drop off from the main stem when their vascular system is plugged.

Breeding efforts to introgress *G. barbadense* resistance into Upland cotton indicate that resistance is multigenic and additive (Wilhelm, 1981). Resistance was reported as incomplete and variably expressed (Bell and Presley, 1969), and heterozygous in some cultivars and lines (Barrow, 1970a). Different levels of resistance to the P-1 strain of *V. dahliae* are best distinguished at a mean temperature of 27°C (Bell and Presley, 1969), and to the P-2 strain at a mean temperature of 22.5°C (Barrow, 1970b). Because of the temperature effects, a plant with a moderate level of resistance to the P-1 strain could be classified as highly resistant at 29°C but susceptible at 25°C. This probably explains why different investigators have concluded, variously, that resistance is dominant, recessive or additive (Bell, 1992). Most studies indicate that resistance in cultivars of *G. hirsutum* is multigenic and can be explained by pooled additive and dominant effects (Devey and Roose, 1987). Barnes and Staten (1961) found that transgressive segregation towards either resistance or susceptibility may occur, and that resistance appears to be quantitative. The high level of resistance in *G. barbadense* is probably the result of two or more genes. When highly susceptible cultivars of *G. hirsutum* are crossed with *G. barbadense*, resistance is incompletely dominant in F_1 hybrids, and segregation in the F_2 and F_3 progeny is not distinct, indicating multiple genes and quantitative inheritance (Bell and Presley, 1969; Wilhelm et al., 1974).

As Pima S-7 and Acala 44 had significant differences in resistance to *V. dahliae*, an interspecific cross was made to develop an F_2 population. Distribution of F_2

Table 4
Number of genes controlling Verticillium wilt resistance, broad-sense heritability and significance of additive effect in the F_2 population

	Number of H. leaves	Number of nodes	Leaf weight	Stem weight	Leaf–stem ratio	Total shoot weight
Mean $P_{R(n=7)}$	7.71	12.71	13.56	7.10	1.91	20.66
Mean $P_{S(n=7)}$	2.43	9.00	2.10	3.57	0.61	5.67
$VarP_R$	0.49	0.49	4.70	0.57	0.06	7.30
$VarP_S$	1.96	0.29	2.64	0.39	0.19	3.03
$VarF_2$	3.55	3.23	13.91	2.72	0.30	25.94
Var_E	1.22	0.39	3.67	0.48	0.13	5.17
Var_G	2.33	2.85	10.24	2.24	0.17	20.77
H^2	0.66	0.88	0.74	0.82	0.58	0.80

Var_E, $VarP_R$ + $VarP_S$)/2]; Var_G, ($Var_{P(F2)}$–Var_E); H^2, (Var_G/Var_P); D, ($MeanP_R$–$MeanP_S$) in F_2; Var_G, genotypic variance; Var_E, environmental variance; P_R, resistant parent; P_S, susceptible parent; $Var_{P(F2)}$, phenotypic variance in the F_2; H^2, broad sense heritability; Var_P, phenotypic variance.

phenotypes indicates that Verticillium wilt resistance is a quantitative trait with some degree of dominance from the resistant parent. Transgressive segregation also was observed.

These results agree with the earlier studies which indicated Verticillium wilt resistance is a quantitative trait controlled by at least two major genes and some minor genes for two mostly used cotton cultivars (Pima S-7 and Acala 44) in gene mapping studies. Results obtained in this study are useful to determine levels of resistance and susceptibility in cotton cultivars and virulence of *V. dahliae* isolates. The parameters, the number of healthy leaves and total shoot weight, are useful to quantify resistance for genetic studies, e.g. developing genetic mapping population in cotton breeding programmes.

References

Adair AS. Screening for tolerance to Verticillium wilt in cotton. MS Thesis. Lubbock, TX, Texas Tech University, 1996, 62 pp.

Barnes CE, Staten G. (1961) The combining ability of some varieties and strains of *G. hirsutum*. New Mexico Agric Exp Stn Bull 457.

Barrow JR. (1970a) Heterozygosity in inheritance of Verticillium wilt tolerance in cotton. *Phytopathology* **60**:301–303.

Barrow JR. (1970b) Critical requirements for genetic expression of Verticillium wilt tolerance in Acala cotton. *Phytopathology* **60**:559–560.

Bell AA. Verticillium wilt. In: Hillocks, RJ (ed.), *Cotton Diseases*. Wallingford, UK, CAB International, 1992, pp. 87–126.

Bell, AA. Biology and ecology of *Verticillium dahliae*. In: Lyda SD, Kenerley CM (eds), *Biology of Sclerotial-Forming Fungi*. College Station, TX, Texas A&M University Press, 1993, pp. 147–210.

Bell AA. Mechanisms of disease resistance in *Gossypium* species and variation in *Verticillium dahliae*. In: Constable GA, Forrester NW (eds), *Challenging the Future: Proceedings of the World Cotton Research Conference-1*, February 14–17, Brisbane, Australia, 1994, pp. 225–235.

Bell AA. Diseases of cotton. In: Smith CW, Cothren JT (eds), *Cotton*. New York, John Wiles and Sons, Inc., 1999, pp. 553–593.

Bell AA, Presley JT. (1969) Temperature effects upon resistance and pyhtoalexin synthesis in cotton inoculated with *Verticillium alba-atrum*. *Phytopathology* **59**:1141–1146.

Blasingame D, Patel MV. Cotton disease loss estimate committee report. In: Herber DJ, Richter DA (eds), *Proceedings of Beltwide Cotton Conference*. San Antonio, TX. Memphis, TN, National Cotton Council, 2004, pp. 459–460.

Bugbee WM, Presley JT. (1967) A rapid inoculation technique to evaluate the resistance of cotton to *Verticillium albo-atrum*. *Phytopathology* **57**:1264.

Carpenter CW. (1914) The Verticillium wilt problem. *Phytopathology* **4**:393.

Devey ME, Roose ML. (1987) Genetic analysis of Verticillium wilt tolerance in cotton using pedigree data from crosses. *Theor Appl Genet* **74**:162–167.

Devey ME, Rosielle AA. (1986) Relationship between field and greenhouse ratings for tolerance to Verticillium wilt on cotton. *Crop Sci* **26**:1–4.

El-Zik KM. (1985) Integrated control of Verticillium wilt of cotton. *Plant Dis* **69**:1025–1032.

Hillocks RJ. (1990) Screening for resistance to Verticillium wilt in Zimbabwe. *Trop Agri* **68**:144–148.

National Cotton Council. Cotton disease loss estimate committee reports. In: Herber DJ, Richter DA (eds). *Proceedings of Beltwide Conference*. Memphis, TN, National Cotton Council, 1980–1999.

SAS Institute. *SAS/STAT User's Guide: Statistics*. Version 6, 4th edn. Cary, NC, SAS Institute, 1994.

Sherbakoff CD. (1928). Wilt caused by *Verticillium alba-atrum*. *Plant Dis Rep Suppl* **61**:283–284.

Wilhelm S. Sources and genetics of host resistance in field and fruit crops. In: Mace ME, Bell AA, Beckman CH (eds), *Fungal Wilt Diseases of Plants*. New York, Academic Press, 1981, pp. 300–369.

Wilhelm S, Sagen JE, Tietz H. (1974) Resistance to Verticillium wilt in cotton: sources, techniques of identification, inheritance trends, and the resistance potential of multiline cultivars. *Phytopathology* **64**:929–931.